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Transformation and Precipitation of Toxic Metals by Pseudomonas maltophilia

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13. ABSTRACT (Maximum 200 words)

The aims of this research are to study each of the various molecular mechanisms whereby toxic metal cations and oxyanions are chemically transformed by Pseudomonas maltophilia strain OR-02. The research effort for the current year has focused on the microbial-dependent transformations of selenite, tellurite, lead, and chromate. The reduction of selenite and tellurite to their insoluble elemental forms appeared to be mediated by an intracellular glutathione reductase that utilized the spontaneously-formed bis(glutathio)Se or bis(glutathio)Te, respectively, as pseudosubstrates. The biomolecules responsible for the extracellular transformation of lead and the reduction of chromate to Cr(III) are currently under investigation. This project could provide useful information toward the eventual exploitation of P. maltophilia and related organisms for the removal of toxic metal wastes from selected, heavily polluted sites.

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A. RESEARCH OBJECTIVES

The aims of this project are to study each of the various molecular mechanisms whereby toxic metal cations and oxyanions are chemically transformed by a remarkable strain of Pseudomonas maltophilia originally isolated from mercury-contaminated soil at Oak Ridge National Laboratory. The specific aims for the current grant period are as follows:

(1) To perform detailed kinetic studies on selected metal transformations using suspensions of intact bacterial cells;

(2) To determine whether each metal transformation is a function of the bacterial cell itself or some exported component(s); and

(3) To identify, separate, purify, and reconstitute the minimum cellular components necessary for metal transformation.

The metal cations and oxyanions to be examined in these investigations include, but are not limited to, Se(IV), Cr(VI), Pb(II), Ag(I), Au(III), Cd(II), Sn(II) and Hg(II).

B. STATUS OF THE RESEARCH EFFORT

The research effort for year 01 of this grant has focused on the microbial-dependent transformations of selenite (SeO_3^{2-} or Se(IV)), tellurite (TeO_3^{2-} or Te(IV)), chromate (CrO_4^{2-} or Cr(VI)), and lead (Pb(II)). Experimental progress to date is summarized below.

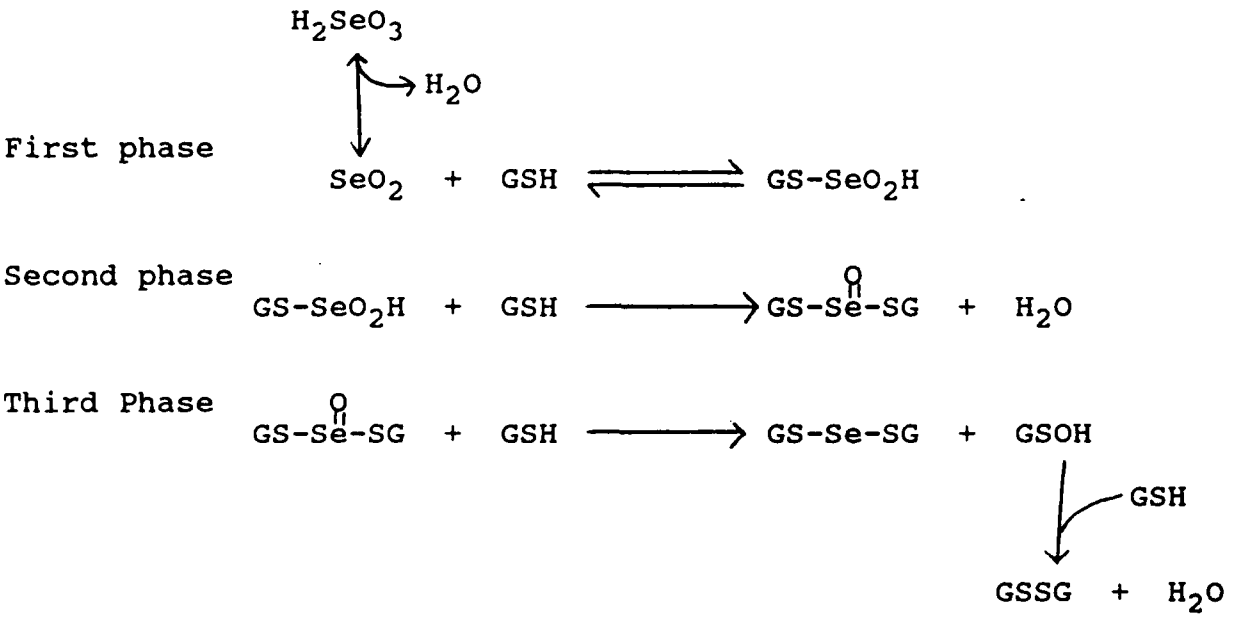
(i) Reduction of selenite

When P. maltophilia (strain OR-02) was grown in nutrient broth containing up to 10 g/l sodium selenite (58 mM), growth of the bacterium occurred concomitantly with the precipitation of the red, elemental form of selenium, Se(0). A representative SEM photograph of strain OR-02 grown in the presence of 40 mM selenite is included as part of Fig. 1. In each SEM photograph of cells of OR-02 exposed to selenite, one can find examples of cells that contain one or more large, electron-dense bodies. These electron-dense bodies are absent in the bacteria that have not been exposed to selenite. The photograph in Fig. 1 contains several bacterial cells in close proximity. The sample in Fig. 1 was further subjected to energy dispersive X-ray spectrophotometry in collaboration with Dr. Larry Barton at New Mexico State University. Dr. Barton works on the reduction of selenite by anaerobic bacteria and has access to sophisticated analytical instrumentation at Los Alamos National Laboratory. The energy dispersive X-ray instrument in question is capable of detecting and identifying any element with an atomic mass greater than that of beryllium. Thus, one could ask what elements were detectable in the portion of the sample defined by the red line in Fig. 1. Sodium, calcium, iron, potassium and phosphorus, all common constituents of any living cell, were shown to be present

throughout the bacterial cells in the figure. The presence of selenium was shown to coincide only with the dark bodies in the photograph. Indeed, by repeatedly repositioning the red line and performing elemental analyses throughout the sample, selenium was shown to be associated exclusively with the dark bodies **within** the bacterial cells.

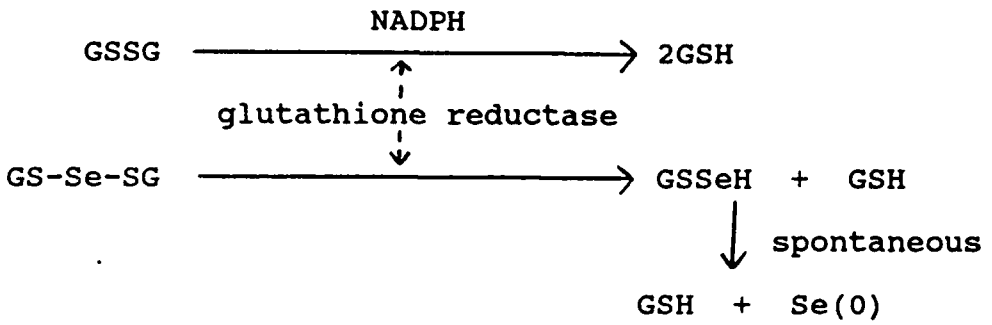
A clue to the molecular mechanism of intracellular selenite reduction was afforded by the bacterial growth studies illustrated in Fig. 2. The flask on the left shows the consequences of culturing OR-02 in nutrient broth containing 40 mM selenite. The abundant red precipitate was visible evidence of the microbial-dependent reduction of the selenite. The flask on the right shows the abundant growth achieved by OR-02 in nutrient broth containing 2.0 mM buthionine sulfoximine (BSO). BSO is an irreversible inhibitor of gamma-glutamylcysteine synthetase and has been widely used to selectively inhibit glutathione biosynthesis without adversely affecting amino acid biosynthesis in general. When both BSO and selenite were present simultaneously, the bacterium would not even grow, much less precipitate elemental selenium (demonstrated by the middle flask in Fig. 2). This growth experiment underscored the importance of glutathione in the protective mechanism(s) adopted by the OR-02 in response to toxic levels of selenite.

Cell-free extracts of OR-02 contained millimolar levels of reduced glutathione (GSH) and detectable glutathione reductase activity. When selenite was rapidly mixed with an excess of GSH in a stopped flow spectrophotometer, the absorbance of the resulting solution underwent three sequential changes consistent with the following chemical changes (data not shown):



The bis(glutathio)selenium, GS-Se-SG, formed as a result of the spontaneous, uncatalyzed reactions introduced above, can then

serve as a reactive substrate analog for glutathione reductase,



resulting in the generation and precipitation of elemental selenium. When NADPH, GSH and selenite were incubated with the soluble fraction of cell-free extracts of OR-02 grown in the presence of selenite, timely precipitation of red, elemental selenium was observed. The omission of NADPH or GSH, treatment of the cell-free extract by proteases or boiling, or the addition of aromatic arsenicals (which inhibit glutathione reductase) all served to strongly inhibit the appearance of red precipitate. Furthermore, the specific activity of the glutathione reductase activity was observed to be 5- to 10-fold higher in extracts prepared from selenite-grown cells when compared to that in extracts prepared from cells grown in the absence of selenite.

The hypothesis constructed from the studies summarized above is that the generation of elemental selenium occurs as a consequence of the glutathione reductase-dependent reduction of the bis(glutathio)selenium that forms spontaneously when selenite and GSH are present together. A manuscript that describes these results is in preparation for submission to the Journal of Biological Chemistry, as indicated below.

(ii) Reduction of tellurite

When OR-02 was grown in nutrient broth containing up to 10.0 mM tellurite, growth of the bacterium occurred concomitantly with the precipitation of the black elemental form of tellurium, Te(0). A series of experiments analogous to those described above are currently underway in which tellurite has been substituted for selenium. The cell growth experiment featured in Fig. 2 produced the same conclusion when tellurite was substituted for selenite (data not shown). Furthermore, we have observed that tellurite and GSH react in a manner similar to that described above to generate bis(glutathio)tellurium, GS-Te-SG. The current working hypothesis is that the generation of elemental tellurium occurs as a consequence of the glutathione reductase-dependent reduction of the bis(glutathio)tellurium that forms spontaneously when tellurite and GSH are present together.

It is anticipated that these experiments featuring the reduction of tellurite will eventually lead to a separate publication.

(iii) Transformation of lead

When OR-02 was grown in nutrient broth containing up to 3.0 mM lead nitrate, growth of the bacterium occurred concomitantly with the appearance of a black precipitate. A combined SEM and energy dispersive X-ray analysis similar to that in Fig. 1 was performed on bacterial cells cultured in the presence of lead nitrate (data not shown). In contrast to the results obtained with selenite, growth of OR-02 in the presence of Pb(II) led to the appearance of dark, electron-dense bodies **outside** of the bacterial cell. These dark extracellular bodies contained all of the detectable lead; lead could not be detected by energy dispersive X-ray spectrophotometry either inside the cell or on the plasma membrane. Furthermore, the presence of BSO had little effect on the growth and lead-transformation activity of the organism in the presence of Pb(II). It has thus become evident that discreet molecular mechanisms exist for the bacterial transformations of Pb(II) and Se(IV), not an entirely unexpected conclusion.

The current goal is to identify the chemical nature of the black precipitate generated when OR-02 is grown in the presence of Pb(II). Samples of the black precipitate were collected and washed exhaustively with hot sodium dodecyl sulfate to remove cellular materials. A washed specimen was subsequently submitted to Surface Science Laboratories, Mountain View, CA, for ESCA analysis (Electron Spectroscopy for Chemical Analysis). The ESCA spectra showed unequivocally that the lead in the black precipitate was not elemental lead. Instead, the lead was cationic, either Pb(II) or Pb(IV). Whatever the cell produces to coordinate this cationic lead, it complexes the lead much more tightly than does EDTA. Chemical analysis of this black precipitate will continue.

In the meantime, we have discovered that the OR-02 appears to express an organolead lyase activity. When grown in the presence of up to 1.0 mM diethyllead dichloride, the OR-02 generated the same black precipitate as that obtained in the presence of mere inorganic Pb(II). Such an organolead lyase activity has never been described in the literature. We intend to examine the apparent substrate specificity of this organolead lyase using whole cells and then attempt to purify the activity from cell-free extracts of the organism.

(iv) Reduction of chromate

Kinetic studies on the microbial-dependent reduction of potassium chromate are currently underway. When cells of OR-02 grown in the presence of chromate were washed and exposed to fresh 1.0 mM or higher chromate, the Cr(VI) species disappeared within 2 hours in an apparent first order process. Over the same time period the reduction product Cr(III) displayed a transient rise and subsequent decay in concentration. The total soluble chromium species thus decayed linearly with the concomitant formation of an off-white precipitate. Whether this light solid represented insoluble Cr(III) trihydroxide (formed as the con-

centration of the Cr(III) species increased) or some further chemical transformation of the chromium remains to be determined. Chemical analysis of this precipitated material will be initiated.

C. PUBLICATIONS

(i) Published - none

(ii) Submitted - none

(iii) In preparation - one, "On the Microbial-Dependent Transformation of Toxic Metals: Mechanism of Selenite Reduction by Pseudomonas maltophilia"; R.C. Blake, D.M Choate, S.H. Bardhan, N.H. Revis, and J.H. Jackson; to be submitted to the Journal of Biological Chemistry

D. PROFESSIONAL PERSONNEL

(i) Postdoctoral Associate - Dr. Ludmilla Weclas-Henderson, a part-time employee for three months

(ii) Research Technician - Donna Choate, employed for 9 months

E. COUPLING ACTIVITIES

(i) Meeting presentations - one, "Reduction of Selenium, Mercury and Lead Salts Encoded by a Single Plasmid in a Pseudomonas Strain from a Toxic Waste Site"; L. Latinwo, L. Overbye, J. Caguiat, R.C. Blake, R. Revis, and J.H. Jackson; presented at the 1990 Annual Meeting of the American Society of Microbiology at Anaheim, CA

(ii) Consultations - none

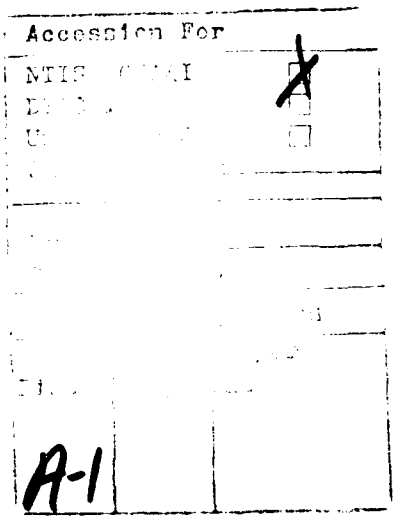
F. NEW DISCOVERIES

The discovery of an organolead lyase activity in strain OR-02, as discussed above.

G. OTHER STATEMENTS

What could be a very productive collaboration has been established between this laboratory and that of Dr. Julius Jackson of Michigan State University. Dr. Jackson is a microbial geneticist who has succeeded in transfering a large plasmid purified from strain OR-02 into a recombination-minus E. coli, thereby transforming the recipient E. coli strain to reduce Hg(II) and Se(IV) and to precipitate Pb(II). Furthermore, Dr. Jackson has obtained transformants that precipitate lead, but do

not reduce selenite, and vice versa. The tentative plan is that Dr. Jackson will send interesting stable transformants to this laboratory for accompanying enzymological experiments. The ability to move various metal transformation activities from one organism to another is a significant advance that broadens the opportunities to study the molecular mechanism(s) of each metal transformation. This ability could also permit the eventual transformation of indigenous bacterial populations already adapted to and inhabiting selected heavily polluted sites.



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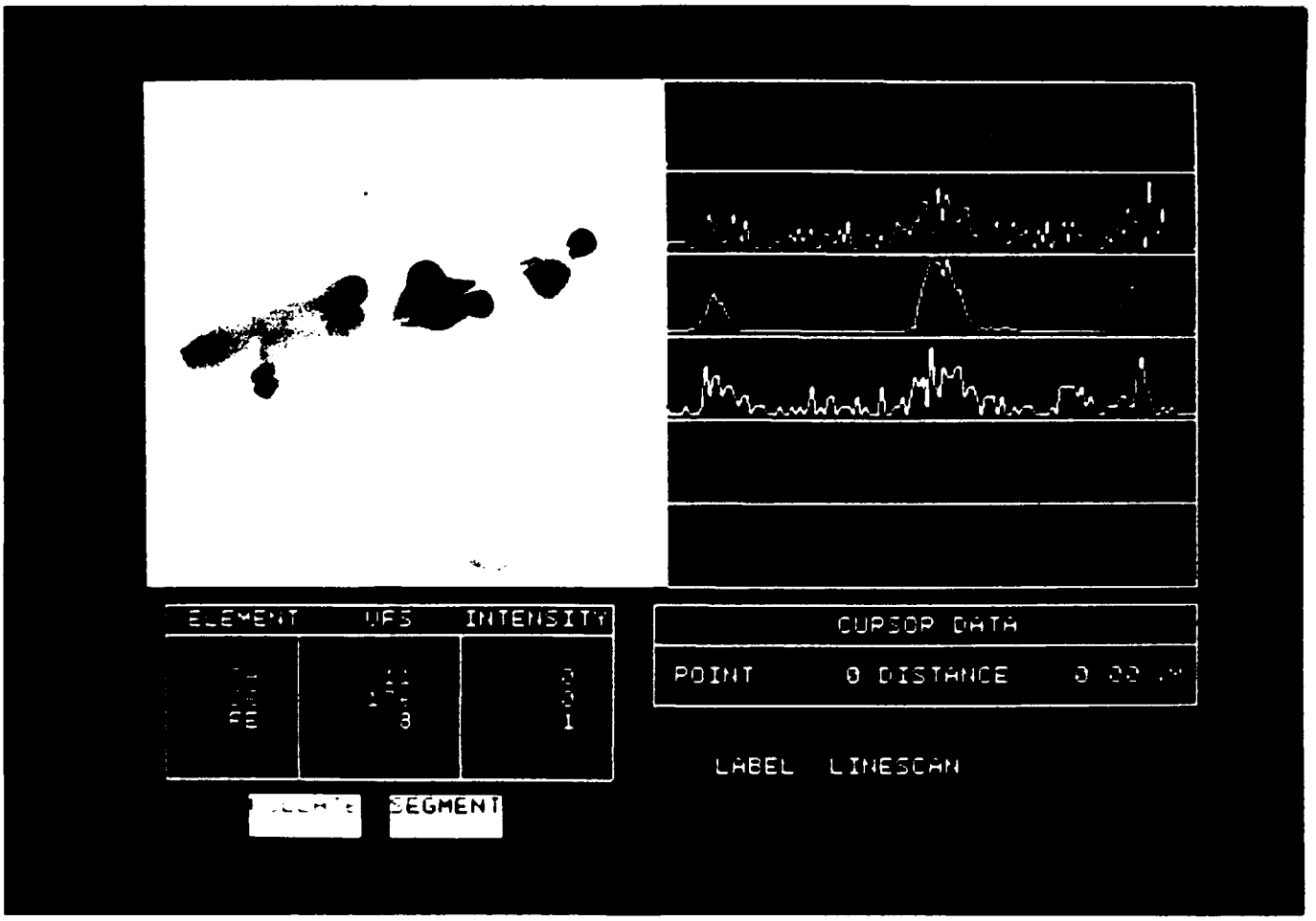

Fig. 1. Representative energy dispersive X-ray analyses of Pseudomonas maltophilia grown in the presence of selenite. Inset, an SEM photograph of P. maltophilia strain OR-02 grown in nutrient broth containing 40 mM sodium selenite. The plots on the right represent elemental analyses of concentration (ordinate axis) versus distance along the red cursor shown in the SEM photograph (abscissa axis). Elements analyzed: sodium, blue; calcium, pink; selenium, orange; iron, yellow; potassium, green; and phosphorus, red.

Fig. 2. Growth of Pseudomonas maltophilia strain ÕR-02 in the presence of selenite (left), buthionine sulfoximine (right), and both selenite and buthionine sulfoximine (middle). All three flasks contained 10 g/l tryptone and 5 g/l yeast extract adjusted to a pH of 7.5. The selenite concentration in the left and middle flasks was 40 mM, while the buthionine sulfoximine concentration in the middle and right flasks was 2.0 mM. Each of the three flasks received the same amount of inoculum and was incubated for 48 hours at 25° C.